THERMAL STUDIES OF ISOXAZOLYL-NAPHTHOQUINONES BY SIMULTANEOUS DTA-TG-DTG
II. Solid-state studies on a ketoenol system

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Abstract

The tautomeric binary system consisting of N-(3,4-dimethyl-5-isoxazolyl)-4-amino-1,2-naphthoquinone (1), m.p. 156°C, and 2-hydroxy-N-(3,4-dimethyl-5-isoxazolyl)-1,4-naphthoquinon-4-imine (2), m.p. 218°C, was studied by DTA-TG-DTG and IR spectrometry. Crystals of 1, left in contact with their mother liquor during 4 months, exhibited thermal behaviour similar to that of physical mixtures of 1 and 2, which reveal the presence of a eutectic at about 146°C.

The effects of light, heat and humidity on the stabilities of 1 and 2 were also studied. Heat (up to 110°C) did not induce any change observable by DTA-TG-DTG in either 1 or 2. Moisture and daylight affected only 1. Upon melting, interconversion of the isomers was not observed.

Keywords: DTA-TG-DTG, keton system, simultaneous, solid-state stability, tautomeric isoxazolylnaphthoquinones

Introduction

It is well established that it is essential to characterize the crystalline nature of every new drug as much as possible during the early phase of preformulation, and also to determine the effects of environmental factors and stress conditions on solids before strategies are developed for their handling, storage and use.

In a previous report [1], we characterized some isoxazolylnaphthoquinones by thermal methods. However, although those compounds were synthesized in 1982 [2], to date no basic study on the effects of degradative factors such as light, heat and humidity has been made in the solid-state.

The aim of the present work was to characterize N-(3,4-dimethyl-5-isoxazolyl)-4-amino-1,2-naphthoquinone (1) by DTA-TG-DTG and to compare its thermal behaviour with that of other isoxazolylnaphthoquinones [1]; to determine if a tautomeric conversion between 1 and its crystalline enol tautomer 2-hydroxy-N-

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(3,4-dimethyl-5-isoxazolyl)-1,4-naphthoquinon-4-imine (2) takes place during melting, as is known for similar compounds [3]; and to study the effects of certain environmental factors on the solid-state stabilities of both isomers.

These derivatives belong in a family of compounds with interesting pharmacological properties. In fact, recent studies [4–7] revealed that 2 displays activity against *Trypanosome cruzi*, the agent responsible for American trypanosomiasis, Chagas' disease [4–6], and also against *Staphylococcus aureus* [7]. In contrast, 1 does not exhibit such activity [5]. This difference in bioactivity highlights the importance of obtaining information on their solid-state stabilities and solid-solid interactions in order to ascertain that 2 is not mixed with 1, and also to define the conditions for reproducible methods of preparation and storage of both compounds.

Experimental

N-(3,4-Dimethyl-5-isoxazolyl)-4-amino-1,2-naphthoquinone (1) and 2-hydroxy-N-(3,4-dimethyl-5-isoxazolyl)-1,4-naphthoquinone-4-imine (2) were prepared and purified to analytical grade by fractional crystallization from benzene- carbon tetrachloride (1) or ethanol (2), following reported procedures [2, 8].

The DTA-TG-DTG analyses were made using a Netzsch simultaneous thermal analyser 429, in N_2 atmosphere, at a heating rate of $10^\circ C$ min^{-1} and a sample mass of 5–10 mg. Empty alumina pans were used as references. The DTA-TG-DTG and DTA-DDTA-TG curves represent the original signals. The IR spectra were recorded in Nujol emulsion with a Nicolet 5 SXC FT IR spectrometer. Physical mixtures of 1 and 2 were obtained by accurately weighing both compounds in various proportions, mixing them by dissolution in the minimum amount of ethanol, and subsequently removing the ethanol by evaporation. The mixtures were then subjected to DTA-TG-DTG and IR.

Samples were irradiated with a Philips Sunlight (220 v/300 w) lamp for 7 days. Heat exposure was performed at $110^\circ C$ in an oven during 46 days. Additionally, samples were exposed to moisture by storage at room temperature in a water-saturated atmosphere of 75% relative humidity (saturated NaCl solution) [9] for 30 days. TLC chromatograms were run on commercial precoated sheets with silica gel and a fluorescent indicator. The spots were visualized with UV and daylight.

Results and discussion

Two different samples of 1, obtained by fractional cystallization (F_1 and F_2), were subjected to DTA-TG-DTG analysis. The DTA plot of F_1 (Fig. 1A), which corresponds to a pure sample, exhibits an endothermic peak at about $165^\circ C$, due to melting, and an exothermic one at about $244^\circ C$, accompanied by mass loss in the simultaneous TG curve, attributable to a decomposition process. These results indicate that 1 melts before decomposing. Therefore, the intermolecular hydrogen-bonding that is assumed to exist in the solid phase [10] seems to be weaker in this compound than in its enol tautomer, which has a free hydroxy group at position 2 of the naphthoquinone ring, and melts with decomposition [1]. Neither solvent nor dehydration peaks are observed for fraction F_1.
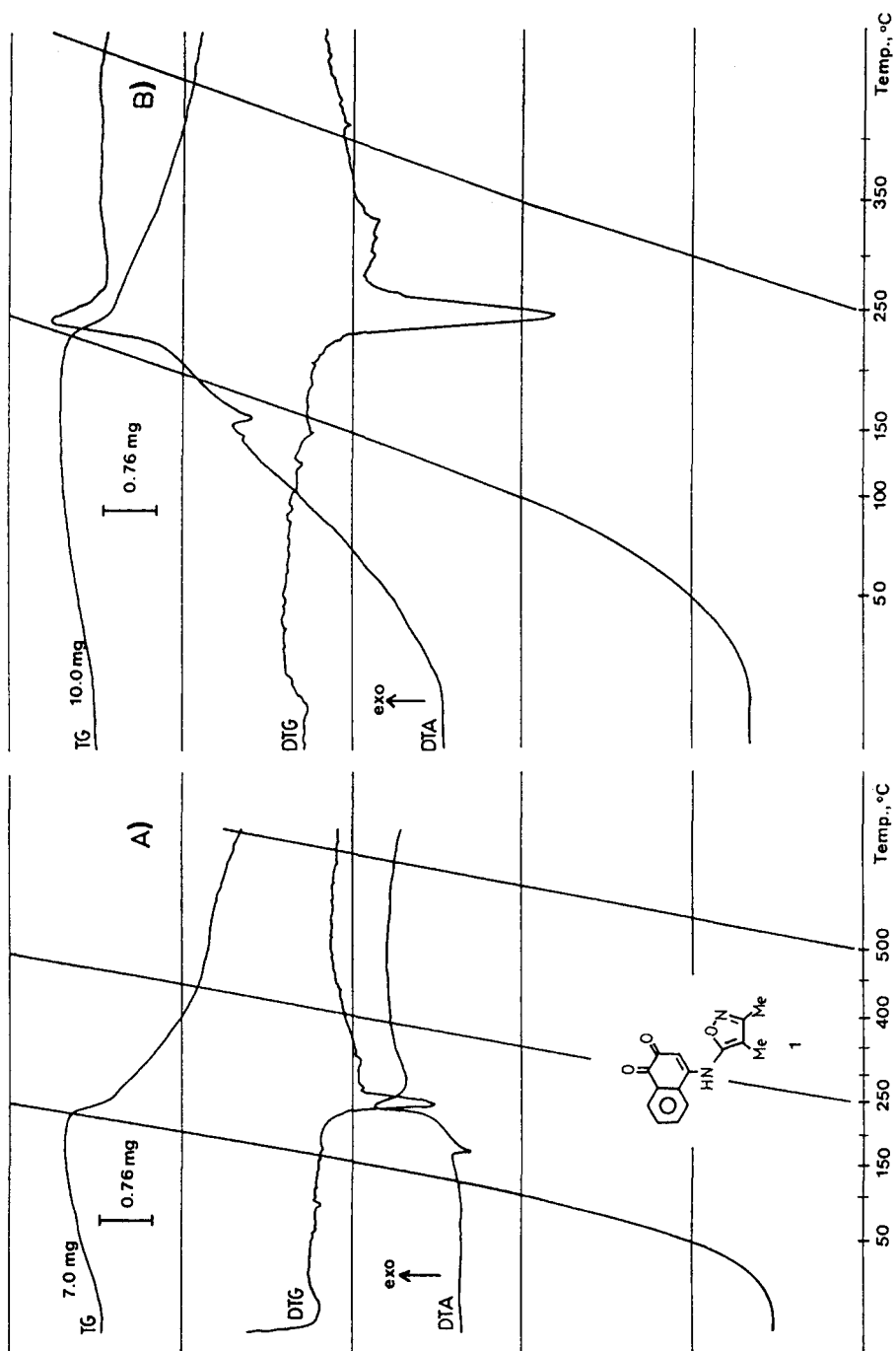
Fig. 1 DTA-TG-DTG curves of N-(3,4-dimethyl-5-isoxazolyl)-4-amino-1,2-naphthoquinone (1) at a heating rate of 10°C min^{-1}: A) F_1. b) F_2

Fraction F_2 (obtained from the liquor mother after 4 months at 5°C) displays an additional small endothermic peak at about 147°C in the DTA curve (Fig. 1B), attributable to a solid-liquid transition, since there is no mass change in the TG curve. HPLC analysis revealed the presence of a second component, which was separated by preparative radial chromatography. The efficiency of this technique was judged from DTA-TG-DTG analysis of the principal component. The endothermic peak at 147 °C was absent; the new DTA-TG-DTG profiles were identical to those of F_1.

With regard to the origin of F_2, and as it is known that 1 tautomerizes to 2 in aqueous and ethanolic solutions [11], the possibility of isomerization to give the enol isomer could not be discounted. Physical mixtures of the two compounds were therefore analysed by DTA-TG-DTG in order to determine whether any type of solid-solid interaction occurred between them, or whether the presence of one isomer as a contaminant of the other could be detected. Figure 2 includes the DTA-DTG or DTA-DDTA traces obtained from pure 1, pure 2 and physical mixtures of them. The six intermediate DTA curves can be seen (Fig. 2B–G) to contain two endothermic peaks. The first, which appears at approximately constant temperature (146±3°C), was not observed in the DTA plots of the pure compounds. The second peak (Fig. 2B–E), which decreased in area with increase of the content of 2 in the blends, corresponds to the melting of 1. The melting peak of 2, which had been obliterated, was discernible from Fig. 2F. This indicates that the resulting plots were not combinations of the heating curves of the simple components, but that an interaction had taken place, giving rise to a trace characteristic of a new entity [12]. According to this, and since the presence of a small peak at the beginning of the initial melting endotherm is indicative of a eutectic solid in a binary system [13–15a], a eutectic was evidently formed. The data on the samples ranging from 79 to 10% of 1 (w/w) show that all begin to thaw at the eutectic temperature, suggesting a lack of solid solution in this composition range [14, 16].

To further substantiate these observations, the IR spectra of two blends of 1 and 2 (Fig. 3C and D) were compared with those of the individual components (Fig. 3A and B). In these spectra, the absorption bands of 1 and 2 can be readily identified. The fact that they were practically unshifted would indicate that no chemical interaction occurs between 1 and 2 [17, 18] and that the eutectic, like other eutectics, consists of a mixture of the pure components [17]. Similarly, the IR spectrum of F_2 (Fig. 3E) does not differ significantly from that of the 79–21% blend of 1 and 2 (Fig. 3C), which confirms the contamination of 1 with 2 as a consequence of a tautomeric conversion in benzene CCl_4 and subsequent eutectic crystallization [19].

During the early stage of this investigation, it was thought that the similarities in the decomposition temperatures of the two tautomers could indicate a conversion of 1 to 2 upon melting, as reported for another keto isomer [3]. In this part of the study, therefore, samples of both compounds were analysed by TLC after heating up to their *m.p.* for 10 min (in a constant-temperature bath). This analysis showed that 1 did not convert to 2 since the two compounds gave different complex mixtures of products. Further, a portion of 1 was heated up to 25°C above its melting temperature in the thermal analyser (Fig. 4A), cooled and reheated up to 281°C
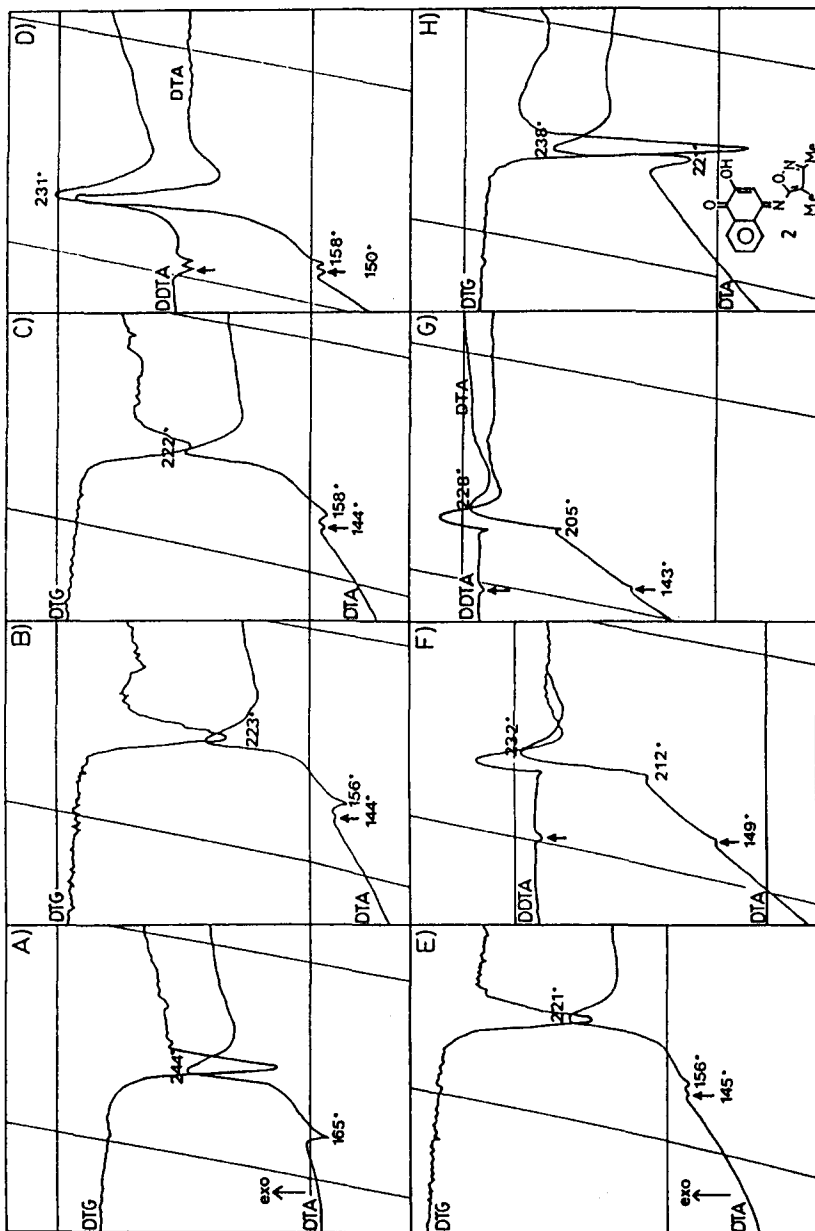
Fig. 2 DTA-DTG or DTA-DDTA curves of: A) pure 1. B) 79-21%, 1-2. C) 58-42%, 1-2. D) 50-50%, 1-2. E) 40-60%, 1-2. F) 20-80%, 1-2. G) 10-90%, 1-2. H) pure 2. (heating rate 10°C min^{-1})
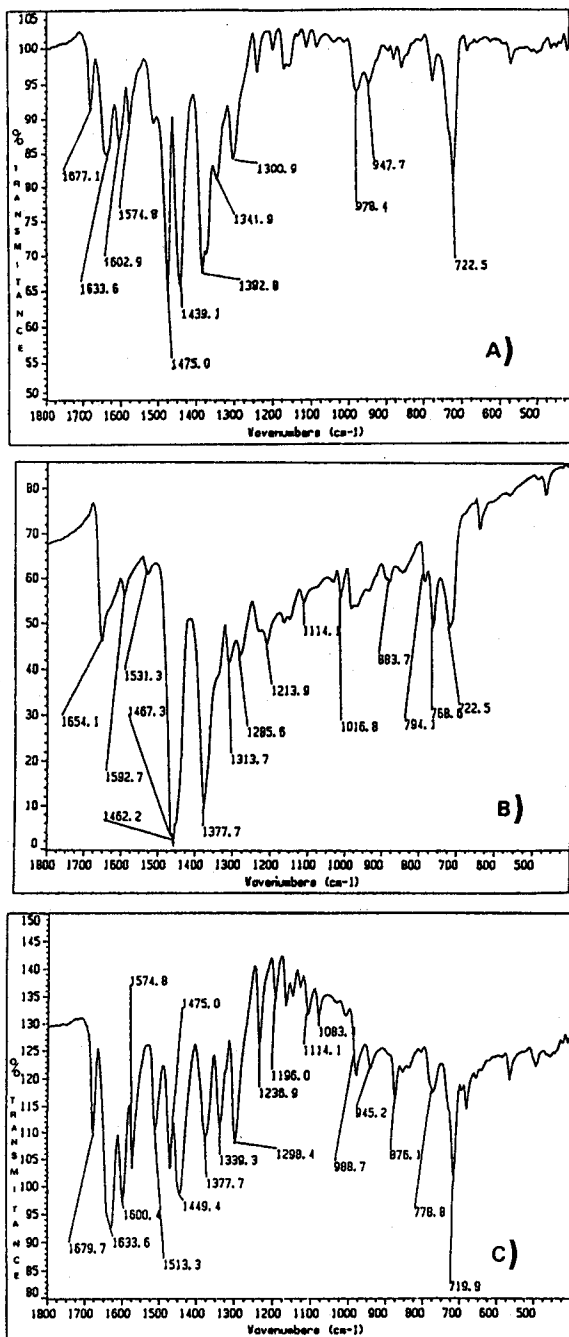
Fig. 3 IR spectra (nujol mull) in the 1800–400 cm^{-1} range: A) pure 1. B) pure 2.
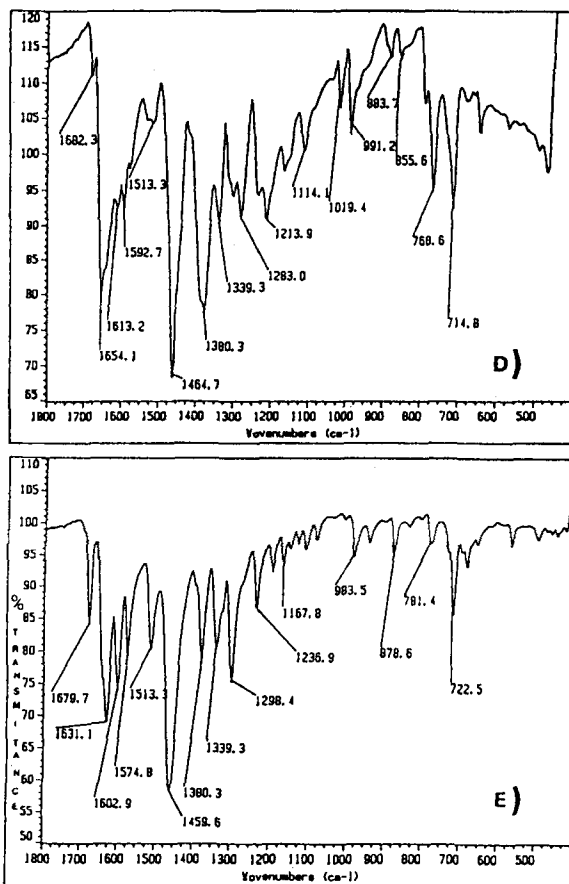C) 79–21%, 1–2

Fig. 3 IR spectra (nujol mull) in the 1800–400 cm^{-1} range: D) 20–80%, 1–2. E) F$_2$

(Fig. 4B). It can be seen that this treatment produced different DTA-DDTA-TG plots; the melting peak disappeared, which clearly indicates the decomposition of 1 above its *m.p.* This result, together with the TLC findings, rule out the possibility of isomer interconversion.

Finally, the solid-state stabilities of 1 and 2 were investigated via DTA-TG-DTG by evaluation of the peak characteristics [15b,c]. Table 1 lists the DTA-DTG thermal data of 1 and 2 after exposure to the environmental factors. The transitions not specifically identified as exo are endothermic.

These data indicate that the keto isomer is stable to heat at 110°C, and to a minor extent to daylight, but it interacts with water vapour. After being kept in the oven, its DTA-DTG data were similar to those of pure 1 (Table 1). No TLC changes were observed. With respect to the light effect, the major phenomenon found was broadening of the melting peak (Table 1), but no decomposition was detected by TLC. After moisture exposure, the DTG curve displayed two extra small endother-

Table 1 DTA-DTG data for 1 and 2 after exposure to environmental factors

Compd.	DTA				DTG		Sample/mg
	onset	peak	onset	peak	onset	peak	
	°C				°C		mg
1	163	165	228	244 (exo)	231	250	7.0
1[a]	164	167	234	242 "	231	248	6.0
1[b]	156	162	226	241 "	228	243	7.5
1[c]	–	–	–	–	86	96	7.5
	–	–	–	–	114	126	
	160	163	–	222 "	192	222	
2	218	221	–	238 "	226	239	10.0
2[a]	222	224	–	242 "	228	242	6.0
2[b]	218	222	–	238 "	226	238	7.5
2[c]	218	223	–	242 "	230	242	7.5

[a] heated at 110°C; [b] irradiated with sunlight; [c] moisture exposure.

mic peaks, with maximum rates at 96 and 126°C, respectively (Table 1), evidently due to water loss. In the corresponding DTA plot, two endothermic effects are barely discernible at these temperatures. Further, broadening and shifting to lower
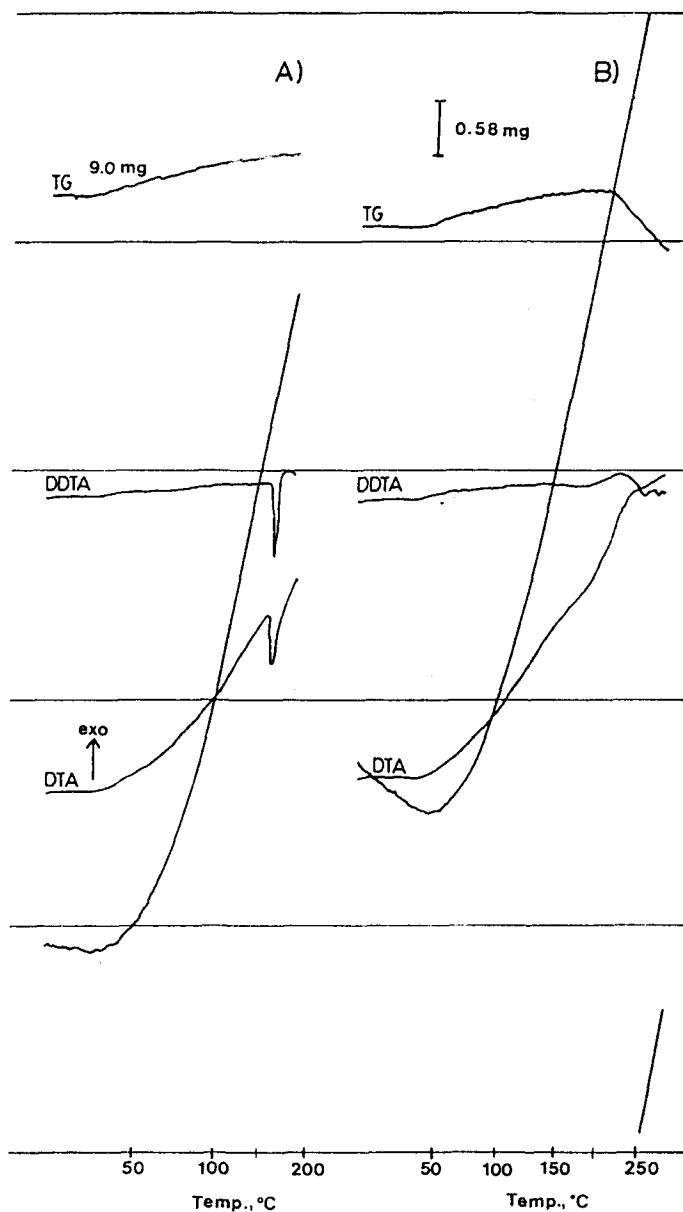

Fig. 4 DTA-DDTA-TG curves of 1 at 10°C min^{-1}: A) first run up to 188°C. B) re-run of the sample after cooling

temperature of the exothermic peak were observed (Table 1), indicating that 1 interacts with water vapour [20]. However, no decomposition was detected by TLC.

In contrast, as shown in Table 1, the enol isomer is quite stable to heat, light and moisture. No significant variation was observed in the DTA-DTG data and no decomposition was detected by TLC.

The present study revealed that 1 and 2 do not tautomerize during melting and that their physical mixtures undergo a eutectic interaction. The evaluation of the solid-state stabilities of 1 and 2, via DTA-TG-DTG, suggests that both isomers are thermally stable below their *m.p.* The keto isomer must be properly protected from humidity and daylight, while it was not necessary to protect 2 from daylight and moisture during manipulation or storage.

<p align="center">* * *</p>

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